

Study Title:

Request for Waivers of Non-Target Plant Protection Studies
for Silver Dihydrogen Citrate Products

Data Requirements:

850.4225: Seedling emergence, Tier II

850.4250: Vegetative vigor, Tier II

850.4400: Aquatic plant toxicity test using *Lemna* spp., Tiers I and II

850.5400: Algal toxicity, Tiers I and II

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Company: ETI H2O, Inc.

Company Agent/Submitter:



Dolana Blount

VP, Product Development/Regulatory Affairs

Date:

09.26.2020

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This volume provides the rationale for waiver requests. The following exposure and risk assessment information is not subject to the principles of the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards as set forth in 40 CFR Part 160.

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Request for Waiver of Non-Target Plant Protection Studies for Silver Dihydrogen Citrate Products

Waivers are requested from the requirements to conduct Non-Target Plant Protection studies (OCSPP 850.4225, 850.4250, 850.4400, and 850.5400) as originally requested by the July 6, 2012 Generic Data Call-In, GDCI-072500-1194, (2012 GDCI) and reinstituted in September 2019. Per the letter from EPA on September 3, 2019, all the ETI H₂O silver dihydrogen citrate (SDC) products have been reclassified from Silver (elemental), PC Code 072501, to Silver ion, PC Code 072500.¹

Introduction & Background

In the 2019 letter, EPA has reinstituted its previous request that that several non-target plant protection studies be submitted on the silver-containing antimicrobial products. Per the 2012 GDCI and 2019 letter, the Agency has requested the following studies:

1. 850.4225: Seedling emergence, Tier II
2. 850.4250: Vegetative vigor, Tier II
3. 850.4400: Aquatic plant toxicity test using *Lemna* spp., Tiers I and II
4. 850.5400: Algal toxicity, Tiers I and II

The footnotes for 850.4225 and 850.4250 specify that these are “required for known phytotoxicants such as herbicides, desiccants, defoliants, and plant growth regulators” (2012 GDCI Footnote 11) and “if a terrestrial species exhibits a 25 percent or greater detrimental effect in Tier 1” (2012 GDCI Footnote 9). For 850.4400 and 850.5400, the footnotes specify that Tier II testing is “required if an aquatic species exhibits a 50 percent or greater detrimental effect in Tier 1” (2012 GDCI Footnote 8). Specifically for 850.4225, the footnotes specify that “Algal Toxicity Tier II data using *Selenastrum capricornutum* (Green Algae), *Anabaena flosaquae* (Blue-Green Cyanobacteria), *Navicula pelliculosa* (Freshwater Diatom), and *Skeletonema costatum* (Marine Diatom) must be submitted to fully satisfy this requirement” (2012 GDCI Footnote 27).

ETI H₂O, a division of PURE Bioscience produces four (4) products containing silver dihydrogen citrate (SDC).² The MUP concentrate (Product 1 below) contains 2400 ppm or 0.240% silver ion and 20.66% citric acid with the remainder consisting of deionized water, produced in an integrated system. Silver *per se* is not present in an isolated form; it exists only as an ion stabilized by citric acid. There is no particulate matter in these products, nor are there any nanoscale particles of silver.³

¹ See letter from EPA to Steptoe & Johnson LLP, dated September 3, 2019. Subject: Amendment to the Generic Data Call-In Notices for Products Containing Silver Particles.

² See 74 FR 27745, July 10, 2009, where the Agency identifies this product as “Silver ions resulting from the use of electrolytically-generated silver ions stabilized in citric acid as silver dihydrogen citrate.”

³ The original DCI requirements from 2012 were placed on hold in 2015 pending an EPA review intended to determine whether existing registered products should be reclassified as “nanosilver.” See letter from EPA from September 3, 2019.

The four registered products are:

1. Axenohl (Reg. No. 72977-1) which is 2400 ppm or 0.240% silver ion and 20.66% citric acid. This is the manufacturing use product (MUP).
2. Axen30 (Reg. No. 72977-3) which is 30 ppm or 0.003% silver ion, 4.8% citric acid
3. Axen50 (Reg. No. 72977-4) which is 50 ppm or 0.005% silver ion, 5% citric acid.
4. SDC3A (Reg. No. 72977-5) which is 30 ppm or 0.003% silver ion, 4.8% citric acid.

The MUP is used solely for making antimicrobial end-use products and ready-to-use sprays and solutions for indoor use (Products 2 through 4 above). Application of the product is achieved via non-aerosol-generating-method (i.e. spray trigger, pump spray, etc.). The directions for use specify that no potable water rinse is needed following application and surfaces can either be wiped with a clean towel or allowed to air dry after sufficient contact time.⁴

The overall weight of evidence rationale for the waivers is as follows:

1. As SDC products are only intended for indoor use only, there is no viable mobility pathway for residual silver to travel into the environment, much less come into contact with a non-target plant, aquatic or otherwise.
2. The SDC products are applied via a non-aerosol-generating-method and do not require wiping the surface or a potable water rinse. As such, very minimal “down the drain” runoff would be anticipated and the vast majority of any remaining residual silver would be removed by wastewater treatment processes.
3. Any extremely small amount of residual silver that would pass through a wastewater treatment plant (WWTP) unchanged and would be removed from the water column rapidly and incorporated into near-by sediment in a non-reactive form (i.e. silver sulfide).
4. EPA recognizes that, specifically for SDC products, the minimal “down the drain” runoff will not result in environmental exposures of concern and no drinking water or ecological risk assessment was needed from the indoor use of SDC products. In fact, EPA has previously concluded that “use of silver as a food contact surface sanitizer will result in minimal, if any, runoff of silver into the surface water. This use will result in an insignificant contribution to drinking water exposures.”⁵
5. The toxicity of silver to plants (aquatic and terrestrial) and algae (freshwater, marine and estuarine) has already been well characterized by both past EPA documents as well as reports found in the open literature. Additional data on algal toxicity would not provide any additional informative value to an ecological risk assessment nor is an ecological risk assessment justified for the indoor uses of SDC products.

Regulatory Basis For Requested Waivers

At 40 CFR 158.45, the Agency identifies that waivers for data requirements can be considered:

⁴ For example, see Directions for Use for Axen 50 (EPA Registration # 72977-4) label dated August 6, 2009.

⁵ See the exemption of tolerance for use of SDC products on food contact surfaces at 74 Fed. Reg. 27451.

a) The data requirements specified in this part as applicable to a category of products will not always be appropriate for every product in that category. Some products may have unusual physical, chemical, or biological properties or atypical use patterns which would make particular data requirements inappropriate, either because it would not be possible to generate the required data or because the data would not be useful in the Agency's evaluation of the risks or benefits of the product. The Agency will waive data requirements it finds are inappropriate, but will ensure that sufficient data are available to make the determinations required by the applicable statutory standards.

The Agency's strategic objective for toxicity testing, as identified at <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science>, clearly identifies the Agency's objective of "Refining and reducing animal testing by maximizing information obtained from animal studies, and focusing on effects of concern" and moving from testing for 'completeness' to carefully focused animal testing where concerns exist, using ... hazard-based hypotheses about the plausible toxicological potential of a pesticide or group of pesticides based on their physical-chemical properties." This is consistent with the National Academies of Sciences recommendations to EPA and is an essential component of the Agency's objectives.⁶

No viable mobility pathway exists for indoor use of SDC products

These products are a MUP for making antimicrobial end-use products and ready-to-use sprays and solutions. All of the products are registered only for indoor use and do not have any outdoor use patterns. Application of the product is achieved via non-aerosol-generating-method (i.e. spray trigger, pump spray, etc.) followed by air drying, or possibly, wiping. The label directions do not require the product be wiped up after application and there is no need for a potable water rinse following application. In fact, for certain use sites (such as meat, poultry and dairy processing plants), the use of a potable water rinse following application is not allowed (for example, see the Axen 50 label).

As such, there is no viable "mobility pathway" for residues on indoor surfaces to reach the outdoor environment, much less the non-target plants and organisms found there. Furthermore, any "down the drain" off-site transport of silver ion would be rapidly transformed to other less mobile and less toxic forms. This is discussed in more detail below.

Silver is mostly removed by WWTPs

The "minimal runoff" associated with indoor use of SDC products could occur in one of two ways: (1) "down the drain" runoff following off-label use (i.e. using a potable water rinse and disposing of rinsate in the sink); (2) or through land application of wastewater sludge biosolids for fertilization purposes. In the former case, the vast majority of free silver ion (as provided by

⁶ The recommendations provided to EPA can be found in the 2007 National Academies of Science/National Research Council report, "Toxicity Testing in the 21st Century: A Vision and a Strategy." Information regarding the implementation of these recommendations can be found here: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science>.

release from SDC) will be removed during the wastewater treatment process, while in the latter case, the species of silver found in biosolids (silver sulfide) poses very low toxicity to plants due to the extremely strong nature of the covalent bond between the silver and sulfide ions.

In considering the effect of down-the-drain disposal of silver-bearing surficial residues, it is useful to consider a “life cycle” approach to follow the fate and transport of silver ion from its application as an SDC spray into the environment.

This can be split into three areas: (1) pre-treatment (from the home to a wastewater treatment plant); (2) treatment; and (3) post-treatment (transformation in the environment following treatment):

(1) Pre-Treatment – In traveling from home plumbing to the WWTP, there is ample opportunity for positively-charged silver cations to bind to negatively-charged anions, to sorb to organic matter in the water column, or to sorb onto some other reactive surface. Silver has the strongest binding preference for reduced sulfur species⁷ such as sulfates (EPA, 2010)⁸, and sulfur in domestic wastewater is both readily available (from urine) and predominantly present as sulfate (Kaegi et al., 2011)⁹. Silver ion also bonds to the ample amount of chloride ions present in natural and waste waters that mix in the sewer pipe, forming insoluble silver chloride. These strong bonds result in immobilization in wastewater and surface waters (Adams and Kramer, 1999)¹⁰. In addition to binding to other ligands, silver ions are also attracted to negatively-charged fulvic and humic acids that are found in organic matter (EPA, 2010)¹¹. Several of the studies cited below demonstrate that the transformation of silver ion into silver chloride and silver sulfate is rapid and occurs prior to the WWTP process.

(2) Treatment – Similar to the opportunities for binding that exist in the waste stream traveling to the WWTP, yet greater opportunities exist at the WWTP itself. Kim et al., (2010)¹² found that silver ion, when bound to sulfur compounds in sludge or other available organic ligands, is much less toxic than free silver ions. Additionally, any silver that sorbs onto available organic matter is highly likely to be removed via agglomeration and precipitation out of the water column (Stensberg et al., 2012)¹³. While the treatment process removes the vast majority

⁷ According to EPA (2010), the solubility product constant for silver sulfides is on the order of 10^{-49} to 10^{-50} , which is roughly five times less soluble than silver chloride (10^{-10}) and over 15 less soluble than silver acetate (10^{-3}). Thus, even in the presence of both sulfate and chloride ions, silver ion has a much greater preference for sulfate over any other anion.

⁸ U.S. EPA. Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray. August 2010. Available at: http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=498022

⁹ Kaegi, R., Vogeline, A., Sinnet, B., Zuleeg, S., Hagendorfer, H., Burkhardt, M. and H. Siegrist. Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. *Environ. Sci. Technol.* 45: 3902-3908, 2011.

¹⁰ Adams, N.W.H. and J.R. Kramer. Silver speciation in wastewater effluent, surface waters, and pore waters. *Environ. Tox. Chem.* 18: 2667-2673, 1999.

¹¹ Ibid., 8

¹² Kim, B., Park, C.S., Murayama, M. and M.F. Hochella. Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products. *Environ. Sci. Technol.* 44: 7509-7514, 2010.

¹³ Stensberg, M.C., Wei, Q., McLamore, E.S., Porterfield, D.M., Wei, A. and M.S. Sepulveda. Toxicological studies on silver nanoparticles: challenges and opportunities in assessment,

of silver¹⁴, it has been reported that a small amount of silver ion can pass through these systems into the aquatic environment (Benn & Westerhoff, 2008)¹⁵. Kaegi et al. (2011)¹⁶ investigated the behavior of silver nanoparticles in a pilot WWTP fed with municipal wastewater and found that most of the silver in both the effluent and sludge was present as silver sulfide and that this reaction occurs rapidly (< 2 hours) under anaerobic conditions. The authors were keen to point out that the rapid transformation time is “considerably shorter than the average hydraulic retention time in the nonaerated tank of the pilot plant (~ 7 h),” implying that there is more than enough residence time in anaerobic tanks of full-scale WWTPs for the “near complete transformation” to silver sulfide. The availability of sulfides (and thus overall sulfidation during the treatment process) was determined by production of sulfide in the anaerobic zones of a sewer biofilm, in an experimental sewer trunk spiked with silver nanoparticles, as reported by Kaegi et al. (2013).¹⁷

Wang et al. (2012)¹⁸ performed a similar study on a number of nanoscale materials (including silver) for a similar duration using processes and retention times more akin to full-scale WWTP. This work confirmed that nanoscale silver will accumulate in biosolids (rather than effluent) as well as demonstrated that nanoscale silver had “negligible effects on ability of the wastewater bacteria to biodegrade organic material, as measured by chemical oxygen demand” (Wang et al, 2012)¹⁹. Schafer et al. (2013)²⁰ reported that silver is eliminated from WWTPs with an efficiency of 95-99% through the rapid transformation (less than 30 minutes) of insoluble silver sulfide mainly attached to flocs of sludge, even when receiving “worst case” influent

monitoring and imaging. *Nanomedicine* (London) 6: 879-898, 2011.

¹⁴ A mass balance exercise for silver in a WWTP published by Shafer et al. (1998) found that over 95% of the influent silver is removed from the water column and not found in the effluent. (Shafer, M.M., Overdier, J.T. and D.E. Armstrong. Removal, partitioning, and fate of silver and other metals in wastewater treatment plants and effluent-receiving streams. *Environ. Tox. Chem.* 17: 630-641, 1998.) Lytle (1984) reported similar removal from WWTPs processing silver-bearing photochemical waste streams. (Lytle, P.E. Fate and speciation of silver in publicly owned treatment works. *Environ. Tox. Chem.* 3: 21-30. 1984.) Kaegi et al. (2011) also performed a mass balance calculation on their pilot WWTP data and determined that only about 5% of the silver left the WWTP in effluent – in this case, this amounted to 7.2 grams of silver out of the influent concentration of roughly 130 micrograms/liter that was fed into the pilot system for over 3 weeks. See Footnote 9. Blaser et al. (2008) modeled the fate and transport of silver ions in the Rhine River and found that the “fraction of silver removed by filtration and treatment” to be 85-99%. (Blaser, S.A., Scheringer, M., MacLeod, M. and K. Hungerbühler. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles. *Sci. Total. Environ.* 390: 396-409, 2008.)

¹⁵ Benn, T.M. and P. Westerhoff. Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol.* 42: 4133-4139, 2008.

¹⁶ Ibid., 9

¹⁷ Kaegi, R. et al. (2013). Fate and transformation of silver nanoparticles in urban wastewater systems. *Water Res.* 47: 3866-3877.

¹⁸ Wang, Y., Westerhoff, P. and K.D. Hristovski. Fate and biological effects of silver, titanium dioxide, and C60 (fullerene) nanomaterials during simulated wastewater treatment processes. *J. Hazard. Mater.* 201-202: 16-22, 2012.

¹⁹ Ibid.

²⁰ Schafer, B. et al. (2013). State of the art in human risk assessment of silver compounds in consumer products: a conference report on silver and nanosilver held at the BfR in 2012. *Arch. Toxicol.* 87: 2249-2262.

concentrations of up to 20 µg/L.²¹ Kaegi et al. (2015)²² reported very low silver concentrations (less than 0.5 µg/L) in the outflow of a WWTP receiving industrial discharge containing silver chloride and silver sulfide nanoparticles, confirming a removal efficiency of greater than 95%. Furthermore, the fraction of silver chloride present in the WWTP influent was almost wholly transformed to silver sulfide in roughly 30 minutes, during sewer transit to the WWTP. There is also recent evidence that the extracellular polymeric substances (EPS), produced by microbes that reside in biofilm and activated sludge, bind silver ion and act as yet another “sink” for any free silver ion that survives the WWTP process.²³

(3) Post-Treatment – Similar to the pre-treatment water column, there is ample organic matter and reduced sulfur species in all aquatic environments to act to reduce any remaining silver ion in WWTP effluent and continue to reduce bioavailability and toxicity to aquatic plants (Luoma, 2008)²⁴. In fact, dissolved sulfides, organic materials and chloride ions will bind up “essentially all the free silver ions in fresh waters (making it unavailable for uptake by organisms) and drive the free silver ions to very low levels” (EPA 2010)²⁵. Silver ions can form complexes with available chloride and ammonium ions to form soluble complexes, covalent adducts with proteins bearing thiol groups (e.g. glutathione, cysteine) and insoluble salt precipitates (like silver chloride, silver sulfide).²⁶ Given that silver is “an extremely particle-reactive metal,” one would expect silver to be “quickly scavenged from the water column, ending up in sediments” in fairly short periods of time (EPA, 2010)²⁷. Most recently, a group of federal researchers conducted a 60-day estuarine mesocosm study using both ionic and nanoscale silver (Cleveland et al. 2012)²⁸ to simulate movement of silver in a “single-level low marsh.” Silver concentrations were then measured via inductively-coupled plasma mass spectroscopy (ICP-MS) in the water column (seawater), sediment, biofilms, hard clams, grass shrimp, mud snails, cordgrass stalks and leaves, and sand. In seawater, silver ion was “completely removed before the first sampling point at 2 h” - which is consistent with the findings of Kaegi et al. (2011)²⁹ described above. Among the biota (both plants and animals), only mud snails and grass shrimp showed any accumulation of silver in their tissues from the mesocosm ion treatment. Both of these species feed on detritus and organic matter and, thus, uptake of silver into these species was from consumption of food items to which silver ion had sorbed (and not from the water column, as there was no measurable ion in the water column). Of particular importance is the fact that, in the ion-treated

²¹ Ibid.

²² Kaegi, R. et al. (2015) Transformation of AgCl nanoparticles in a sewer system – A field study. *Sci. Total Environ.* DOI: 10.1016/j.scitotenv.2014.12.075.

²³ Geyik, A.G. and F. Cehen (2016). Exposure of activated sludge to nanosilver and silver ion: inhibitory effects and binding to the fractions of Extracellular Polymeric Substances. *Bioresource Tech.* DOI: 10.1016/j.biortech.2016.03.157.

²⁴ Luoma, S.N. Silver nanotechnologies and the environment: Old problems or new challenges. Project on Emerging Nanotechnologies, 2008.

²⁵ Ibid., 8

²⁶ Ibid., 20

²⁷ Ibid., 8

²⁸ Cleveland, D., Long, S.E., Pennington, P.L., Cooper, E., Fulton, M.H., Scott, G.I., Brewer, T., Davis, J., Petersen, E.J. and L. Wood. Pilot estuarine mesocosm study on the environmental fate of silver nanomaterials leached from consumer products. *Sci. Total Environ.* 421-422: 267-272, 2012.

²⁹ Ibid., 9

mesocosm, there was no accumulation of silver into biofilms, sediment or sand. The authors speculate that lack of anaerobic conditions at the water-sediment interface or short residence in the water column prevented transfer of silver ion into sediments, as would be expected given the organic matter and ligand content. Overall, the mesocosm study shows very low potential for accumulation in estuarine biota from the water column and that exposure to aquatic organisms would mainly be expected through consumption of silver-bearing food items. In addition, the study confirms the rapid removal of free silver ion from the aquatic environment, thus minimizing the exposure potential to aquatic organisms.

In fact, the EPA agrees with this assertion, according to the justification it provided in granting an exemption from a tolerance for food-contact uses of SDC products. In addition to FIFRA registration, the SDC products have received an exemption from tolerance (74 FR 27447, June 10, 2009). This was granted specifically to silver ions stabilized with citric acid as SDC, and not extended to “any other silver-containing compounds whether they are other silver salts, complexes with inorganic polymers such as zeolites, or metallic silver in any form or dimension including nanoscale.” Beyond reviewing the low mammalian toxicity of SDC, in granting the tolerance exemption, EPA recognized that, “[T]he uses identified as indoor hard surface applications will result in minimal, if any, runoff of silver into the surface water. The use of silver as a food contact surface sanitizer will result in minimal, if any, runoff of silver into the surface water. This use will result in an insignificant contribution to drinking water exposures.” (74 Fed. Reg. at 27451). In asserting that “minimal, if any” runoff will occur as a result of indoor uses of SDC products, EPA agrees that this use pattern and associated label instructions is unlikely to lead to environmental exposures of concern.

The phytotoxicity of silver is well-characterized

The toxicity and mechanism of silver ions to algae has been well characterized, in both EPA documents and the available open literature. In general, adverse effects of silver ion on aquatic plants “would not be expected under natural environmental conditions because silver can be readily transferred into biologically nonreactive compounds. Sulfides, dissolved and particulate organic matter, chloride, and enzymes within the biota have all been shown to reduce the toxicity of ionic silver” (Ratte, 1999³⁰; Levard et al., 2012³¹).

No-effect concentrations were reported at 2 and 0.002 mg/L for freshwater and marine algal species, respectively (Ratte, 1999)³². Several of these studies are listed in the recent review published by Fabrega et al. (2011)³³ and/or in Table 6 (starting on page 21) of Eisler (1996)³⁴. Data exists on the acute toxicity of silver for both freshwater and marine algal species. In

³⁰ Ratte, H.T. Bioaccumulation and toxicity of silver compounds: a review. *Environ. Tox. Chem.* 18: 89-108, 1999.

³¹ Levard, C., Hotze, E.M., Lowry, G.V. and G.E. Brown. Environmental transformations of silver nanoparticles: impact on stability and toxicity. *Environ. Sci. Technol.* Epub ahead of print, February 29, 2012.

³² Ibid., 30

³³ Fabrega, J., Luoma, S.N., Tyler, C.R., Galloway, T.S. and J.R. Lead. Silver nanoparticles: behavior and effects in the aquatic environment. *Environ. Int.* 37: 517-531, 2011.

³⁴ Eisler, R. Silver Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. National Biological Service, US Department of the Interior. Biological Report 32. September 1996.

presenting its rationale for an ambient water quality criterion of 50 µg/L for silver, EPA lists many studies on algal species in Table 4 (EPA 1980, at B-26 and B-27). Table 4 of the EPA ambient water criteria document also lists several prior studies on freshwater algae, including blue-green, green and diatoms.³⁵ Inhibition of growth was found to be the most sensitive endpoint across all the species considered, occurring as low as 50 µg /L for silver nitrate in the freshwater green alga, *Scenedesmus obliquus* (Bringman & Kuhn 1959)³⁶. EPA (1978) reported 96-hour EC₅₀ values for the marine diatom *Skeletonema costatum* in the range of 130-170 µg /L.

Salas et al. (2019) reported the effects of ionic silver (from silver chloride) on the photosynthetic yield of *Chlamydomonas reinhardtii* (green algae), using doses of 50 to 7500 nM.³⁷ Silver ion from silver chloride affected photosynthetic activity in a dose-dependent manner and the EC₅₀ was reported to be 779 nM. Similarly, Navarro et al. (2015)³⁸ reported the EC₅₀ at 1 hr as 0.18 µM for disruption of photosynthesis by silver ion (as silver nitrate) in *Chlamydomonas reinhardtii*. Taylor et al. (2016)³⁹ investigated the magnitude and mechanism of silver ion toxicity to *Chlamydomonas reinhardtii* (green algae) and a cyanobacterium (*Synechococcus leopoliensis*) and found that ionic silver significantly affected cell viability for both species (cyanobacterium was more sensitive) and that ROS production was significantly affected in the green algae. EC₅₀ values for photosynthetic yield following silver nitrate exposure were reported to be 21.25 – 31.94 µg/L

Thus, EC₅₀ values for freshwater and marine algal species has already been determined and 850.4225 would not provide any additional data for use in risk assessment.

There is publicly available data showing that silver is phytotoxic to *Lemna* spp., including relevant information from the EPA ambient water quality criteria for silver. Brown & Rattigan (1979)⁴⁰ reported on the phytotoxicity of silver nitrate for three freshwater macrophytes. Concentrations of 100 µg /L and higher inhibited the evolution of oxygen in *Elodea canadensis* (waterweed) while complete phytotoxicity occurred at 7,500 µg /L. Concentrations of 270 µg /L and higher caused total phytotoxicity in *Lemna minor* (duckweed). Xu et al (2010) reported on the acute phytotoxicity of silver (as silver nitrate in a nutrient medium) to a submerged rooted freshwater macrophyte, *Potamogeton crispus*. All dosage levels of silver nitrate demonstrated physiological, biochemical and ultrastructural effects, with the chloroplast and mitochondria as

³⁵ Additional silver ion data for algae, phytoplankton and diatoms is reported in the 2019 book chapter by Domingo, G. et al. (2019) Phytotoxicity of Silver Nanoparticles to Aquatic Plants, Algae, and Microorganisms. Chapter 8 in *Nanomaterials in Plants, Algae and Microorganisms*. Elsevier, Inc. 2019.

³⁶ Bringmann, G. and R. Kuhn. Comparative water-toxicology investigations on bacteria, algae and daphnids. *Ges. Ind.* 80: 115, 1959.

³⁷ Salas, P. et al. (2019). The role of size and protein shells in the toxicity to algal photosynthesis induced by ionic silver delivered from silver nanoparticles. *Sci. Total Env.* 692: 233-239.

³⁸ Navarro, E., Wagner, B., Odzak, N., Sigg, L., & Behra, R. (2015). Effects of Differently Coated Silver Nanoparticles on the Photosynthesis of *Chlamydomonas reinhardtii*. *Environmental Science & Technology*, 49(13), 8041–8047. doi:10.1021/acs.est.5b01089.

³⁹ Taylor, C. et al. (2016). Toxic interactions of different silver forms with freshwater green algae and cyanobacteria and their effects on mechanistic endpoints and the production of extracellular polymeric substances. *Environmental Science Nano.* : DOI: 10.1039/c5en00183h.

⁴⁰ Brown, B.T. and B.M. Rattigan. Toxicity of soluble copper and other metal ions to *Elodea canadensis*. *Environ. Pollut.* 20: 303, 1979.

major toxicity targets. [The lowest dose was 5 micromolar (μM) silver nitrate.] Additional silver ion data for *Lemna minor* and other aquatic plants are reported by Domingo et al. (2019)⁴¹.

Thus, toxicity data specifically for *Lemna* spp., as well as other aquatic plants, has already been determined and data from 830.4400 would not hold any additional informative value.

Furthermore, given that the data from 830.4400 is not used directly in the risk assessment, it is superfluous to the continued registration of SDC-based products.

There is also publicly available data on the toxicity of silver to terrestrial plants. Plant species plays a significant role in the transport of silver ion and monocot species more readily transport silver ion to shoots than dicot species. In experiments with soybean and wheat plants, it was found that over 97% of silver ion accumulated in the cell wall fraction, likely due to numerous negatively-charge sites on the plant cell wall.⁴² Once taken up by plants, silver ions can affect photosynthesis through competitive substitution of copper (Cu^+) in plastocyanin, which is a copper-binding protein produced in chloroplasts and acts as an electron carrier in the photosynthetic electron-transfer chain.⁴³

Hirsch (1998)⁴⁴ reported on the effects to agricultural crops (which were grown from seed) upon treatment with sewage sludge-amended soils containing up to 106 mg/kg of silver (as silver sulfide). The growth rate and yield of corn, oat and turnip plants was unaffected and, in fact, yields for lettuce, oat, turnip and soybean were higher in sludge-amended soils. Soils with concentrations of 14 mg silver sulfide/kg soil did adversely affect growth in Chinese cabbage. The silver concentrations in the edible portions of all crops tested did not exceed 80 parts per billion dry weight, except lettuce which contained 2.7 parts per million dry weight after being grown in soils with 120 mg silver per kg soil. Smith and Carson (1977) reported that sprays containing silver killed corn, tomato and bean plants at doses anywhere from 10 to 1,000 mg dissolved silver per liter; however, Wallace and Mueller (1980) report that bush bean plants severely damaged by silver nitrate application in a nutrient solution recovered “at least partially after the [silver] had been removed from the nutrient solution.” In addition, terrestrial plants do not readily accumulate silver from soils, even when grown in high silver concentrations, such as the soil from silver mine tailings (Ratte 1999).⁴⁵ Based on the small amount of data available on terrestrial plants, it appears that silver is not permanently phytotoxic and does not impact the emergence of seedlings or vegetative vigor of important agricultural crops at fairly high levels of exposure (over 100 parts per million).

The registrant also requests a waiver from both 850.4225 (Seedling emergence) and 830.4250

⁴¹ Additional silver ion data for algae, phytoplankton and diatoms is reported in the 2019 book chapter by Domingo, G. et al. (2019) Phytotoxicity of Silver Nanoparticles to Aquatic Plants, Algae, and Microorganisms. Chapter 8 in Nanomaterials in Plants, Algae and Microorganisms. Elsevier, Inc. 2019.

⁴² Quah, B. et al. (2015). Phytotoxicity, uptake and accumulation of silver with different particle sizes and chemical forms. *J. Nanopart. Res.* 17:277.

⁴³ Yan, A. and Z. Chen (2019). Impacts of silver nanoparticles on plants: A focus on the phytotoxicity and underlying mechanism. *Int. J. Molec. Sci.* 20(5). pii: E1003. doi: 10.3390/ijms20051003.

⁴⁴ Hirsch, M.P. Availability of sludge-borne silver to agricultural crops. *Environ. Tox. Chem.* 17: 610-616, 1998.

⁴⁵ Ibid., 30

(Vegetative vigor) as, because there is no viable silver ion exposure pathway to terrestrial plants from indoor SDC product use, there is no need to run ecological risk assessment scenarios for terrestrial plants. Therefore, seedling emergence and vegetative vigor data would not be of use. Additionally, the EPA's final work plan for silver mentions that an ecological risk assessment will only be conducted for outdoor applications of silver compounds.

Implications for risk assessment

It is important to note that Risk Assessment and Science Support Branch of the Antimicrobials Division has specifically stated that indoor hard surface applications of SDC product Axen 50 are “anticipated to result in minimal, if any, runoff of silver into groundwater.”⁴⁶ It is for this reason that AD found a drinking water assessment unwarranted for Axen 50 “based on the uses proposed on this label.” Furthermore, the Axen 50 label specifies that “potable water rinse is not permitted following the use of this product as a sanitizer” on non-porous hard surfaces in meat, poultry and dairy plants to prevent runoff at these use sites. In fact, AD considers the subsequent dietary exposures from food processing equipment sanitization so “extremely small that it is considered negligible and not included in the combined or aggregate [dietary] assessments.”

The 2009 Final Work Plan for silver and silver compounds specified that an ecological risk assessment would only be conducted for outdoor residential use sites, such as “materials preservative use of siding for housing and stucco; and coatings/films for siding for housing, roofing, shingles, building materials, wood and plastic composites, concrete, cement, and glazes for cement.”⁴⁷ It was stated in the 2009 summary of environmental fate and ecotoxicity data for silver compounds that the “Agency has an adequate ecological toxicity data base for silver and silver salts. Therefore, these data are adequate for assessing risk from potential ecological exposures to elemental silver and silver salts.”⁴⁸

EPA assumptions for “worst-case” residual active ingredient on a surface following application is to assume 1 milligram of SDC solution per square centimeter (cm²). This would be adjusted for the level of silver ion in that 1 milligram of SDC solution (e.g. up to 0.005%, or 50 ppm silver ion maximum), or 0.005 mg silver ion/cm² at worst. As explained above, these small amounts of silver ion would, if released to the environment, encounter the ubiquitous amounts of sulfide and chloride available in the natural world and be readily converted to much more stable silver sulfide or silver chloride. The presence of sulfur and chloride is not only associated with a decrease of silver ion in the environment but a reduction in toxicity to aquatic plants and animals, including *Lemna minuta*.⁴⁹ However, it is important to note that “down the drain” exposure could *only* occur through off-label use, such as a potable water rinse following application.

⁴⁶ Memorandum dated July 21, 2008 from Talia Lindheimer (RASSB/AD) to Marshall Swindell (RMBI/AD). Subject: Revised Dietary, Drinking Water Residential and Occupational Exposure Assessment for the Proposed New Uses of Silver (Axen 50 EPA Reg # 72977).

⁴⁷ See the 2009 Registration Review Final Work Plan at www.regulations.gov, Docket EPA-HQ-OPP-2009-0334-0023.

⁴⁸ Memorandum dated June 4, 2009 from Najm Shamim (RMBII/AD) to Marshall Swindell (RMBI/AD). Subject: Summary of Product Chemistry, Environmental Fate, and Ecotoxicity Data for Silver, Silver Salts, Silver Zeolites (Copper and Zinc) and Silver Sodium Hydrogen Zirconium Phosphate for Registration Review.

⁴⁹ Levard, C. et al. (2013). Sulfidation of silver nanoparticles: natural antidote to their toxicity. *Env. Sci. Tech.* 47(23):13440-8. doi: 10.1021/es403527n. Epub 2013 Nov 15.

Given the low likelihood of off-site transport of silver ion from indoor use of SDC products and the environmental transformation that silver ion undergoes before, during and after the WWTP process, EPA has previously obviated the need for drinking water and ecological risk assessments for SDC products. Additional data on algae or aquatic/terrestrial plants would therefore be superfluous.

Conclusions

ETI H2O is requesting the following studies, as requested under the Registration Review GDCI, be waived:

1. 850.4225: Seedling emergence, Tier II
2. 850.4250: Vegetative vigor, Tier II
3. 850.4400: Aquatic plant toxicity test using *Lemna* spp., Tiers I and II
4. 850.5400: Algal toxicity, Tiers I and II

ETI H2O is requesting the waivers for the following reasons:

1. As SDC products are only intended for indoor use only, there is no viable mobility pathway for residual silver to travel into the environment, much less come into contact with a non-target plant, aquatic or otherwise.
2. As SDC products are applied via a non-aerosol-generating-method and do not require wiping the surface or a potable water rinse, very minimal “down the drain” runoff is anticipated and the vast majority of any remaining residual silver would be removed by wastewater treatment processes.
3. Any extremely small amount of residual silver that would pass through a wastewater treatment plant (WWTP) unchanged would be removed from the water column rapidly and incorporated into near-by sediment in a non-reactive form (i.e. silver sulfide).
4. EPA recognizes that, specifically for SDC products, the minimal down-the drain runoff will not result in environmental exposures of concern and no drinking water or ecological risk assessment was needed from the indoor use of SDC products. In fact, EPA has previously concluded that “use of silver as a food contact surface sanitizer will result in minimal, if any, runoff of silver into the surface water. This use will result in an insignificant contribution to drinking water exposures.”⁵⁰
5. The toxicity of silver to plants (aquatic and terrestrial) and algae (freshwater, marine and estuarine) has already been well characterized by both past EPA documents as well as reports found in the open literature. Additional data on algal toxicity would not provide any additional informative value to ecological risk assessment nor is an ecological risk assessment justified for the indoor uses of SDC products.

⁵⁰ See the exemption of tolerance for use of SDC products on food contact surfaces at 74 Fed. Reg. 27451.